VITAMIN CONTENT OF BUTTERS

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VITAMIN CONTENT OF AUSTRALIAN, NEW ZEALAND AND ENGLISH BUTTERS

By M. E. F. Crawford, E. O. V. Perry and S. S. Zilva

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PREFACE

THIS Report gives an account of investigations forming part of a scheme of research into the vitamin content of foodstuffs, particularly fruits, vegetables, and dairy products, and into the effects upon it of different methods of cultivation, preparation, transport, and storage. These studies are made by Dr. S. S. Zilva and his assistants, working in the service of the Medical Research Council at the Lister Institute, London, with valuable collaboration by workers in the Dominions. A grant for the cost of the work during a period of years was made to the Council by the Empire Marketing Board.

Accounts of other parts of these investigations have already been published, either in monograph form or as papers contributed to scientific journals, and the present report deals only with the work done on the vitamin content of butters of Empire origin. The subject has importance in that butter, among ordinary foodstuffs, is at its best a particularly good source of the fat-soluble accessory food factors, vitamins A and D, which are essential for the normal growth of children and for the maintenance of good health. Vitamin A plays a part in maintaining resistance to infections, while vitamin D is necessary for the proper formation of the bones and teeth, and a deficiency of it causes rickets in children and gives increased liability to dental decay. Potency in respect of these two factors is therefore an extremely important quality in butter, and the fact that butters marketed in this country come from many different sources made it desirable to investigate the influence upon their vitamin content brought by the varying conditions of production, transport, and distribution.

From a practical point of view, it is gratifying to find from the Report that the butters imported from Australia and New Zealand show a high and uniform potency in vitamins A and D. They are equal in this respect to the best summer butters produced at home, and even to butter made from the milk of cows whose diet has been fortified by an artificial supply of the vitamins. The finding is the more important in that these butters from the southern hemisphere are available in our winter, when it is known that the vitamin content of milk and butter produced in northern latitudes falls below its summer level. Butter from the southern Dominions, moreover, constitutes an increasing proportion of our total supply: in 1913 some 20 per cent. of the United Kingdom imports of butter came from the southern Dominions, while in 1927 the proportion had increased to 30 per cent., and last year to no less than 44 per cent.

It is also satisfactory to note that the fat-soluble vitamins in butter have great stability in cold-storage. There is, indeed, no fear of sig-

nificant loss in potency under the prevailing conditions of shipment and distribution.

Other practical points have emerged. The breed of the dairy herd seems to be unimportant in this regard. Butters from different parts of Australia, with widely divergent conditions of climate and pasture, showed little variation. The process of neutralizing the acid creams before churning was found to have no destructive effect.

MEDICAL RESEARCH COUNCIL,
38 Old Queen Street,
Westminster, S.W.1.

4th October, 1932.

THE VITAMIN CONTENT OF AUSTRALIAN, NEW ZEALAND AND ENGLISH BUTTERS

BY

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(From the Division of Nutrition, Lister Institute, London.)

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I. INTRODUCTION

The importance of dairy products in the nutrition of the human race need not be stressed. There is scarcely a people, whether primitive or civilized, which has not included them in its dietary from time immemorial, and few articles of food meet so many of the nutritional requirements of the human organism.

Milk contains all the known vitamins, and of these vitamins A and D are present in the fat. As no very significant loss of the fat-soluble vitamins is incurred in the usual process of separating and churning milk-fat, almost the entire fat-soluble vitamin content of the milk is to be found in the butter (Crawford, Golding, Perry, and Zilva, 1930).

Butter even at its best cannot rival cod-liver as a source of vitamins A and D, or mammalian liver as a source of vitamin A, or still less irradiated ergosterol as a source of vitamin D. It must, however, be borne in mind that it is consumed in moderately large quantities and at regular intervals; its function is therefore preventive rather than curative, and as such its value as a vitamin food is high. As far as this country is concerned this dietetic property of butter assumes special importance in the winter when the amount of the fat-soluble vitamins, especially D, consumed in the general diet may often fall short of the minimum requirements, and thus the consumption of a potent butter can be instrumental in making such diet adequate in this respect.

The fact that butters consumed in this country are of many origins and are prepared under different conditions suggested the necessity of investigating the influence of the various factors connected with their manufacture, transit, and distribution on their vitamin content. Our present knowledge concerning this problem is based mainly on theoretical considerations and scattered items of experimental evidence obtained by the use of a variety of methods at different periods of the development of the subject. Such information obviously cannot be considered entirely satisfactory from the point of view of preventive medicine.

In this investigation an attempt was made to study the various points systematically, and the results dealing with English, Australian, and New Zealand butters only are reported. From the beginning of the work it was realized that many difficulties were associated with it, especially as regards the methods of determination of the vitamins. It was considered that the methods suitable for an investigation of this character should be biological and of an objective character so that the results when recorded would be open to the interpretation of the reader as well as of the experimenter. Such methods are laborious and slow, and even when practised under the most carefully controlled conditions do not yield results of very high accuracy. They have the further disadvantage that the number of doses that can conveniently be employed in each test is limited, so that the minimum dose, which in

this inquiry was intended to serve to a great extent as the basis for comparison, may sometimes not be attained. Nevertheless, although small differences in vitamin activity cannot thus be established, any great disparity in potency such as might be indicative of a serious loss in the vitamins, can undoubtedly be registered by the methods employed. It is also to be pointed out that owing to some factors still unknown and consequently not yet controlled, the response of the experimental animals to substances of the same potency may fluctuate somewhat when tested at different times. The magnitude of such fluctuations, in the case of tests carried out in the same laboratory under carefully controlled conditions, would, in our opinion, most probably fall outside the range of accuracy which could justifiably be aimed at by the methods used. It might even be considered as doubtful whether the utilization of a notation of units based on standards such as have recently been adopted by the League of Nations and which were not available at the inception of the inquiry, would have served as a better basis of comparison in these circumstances.

II. HISTORICAL SURVEY

A more complete understanding of the results reported in this publication will be obtained if an historical survey of the development of our knowledge of the fat-soluble vitamins A and D in butter is briefly outlined. It will be sufficient for this purpose to mention the salient facts, starting with the discovery of the fat-soluble complex which is now known to contain vitamins A and D. This physiological entity was revealed by the work of McCollum and Davis (1913) and of Osborne and Mendel (1913, 1 and 2). The former workers discovered that when rats ceased to grow on an artificial diet of casein, lactose, lard, and inorganic salts a resumption of growth could promptly be induced by the introduction into the diet of an ether extract of egg-yolk or butter. As a result of their researches they postulated the existence in these products of some organic complex indispensable for growth, which they designated later as the 'fat-soluble A factor'. Osborne and Mendel arrived independently at a similar conclusion. The discovery of the 'fat-soluble A factor' or the 'growth-promoting factor', or 'vitamin A' as it was afterwards called, led to an extensive study by various workers of its distribution in nature. Further facts concerning its physiological function also came to light. It was revealed very soon after its discovery, for instance, that rats subsisting on a diet deficient in this factor were subject to respiratory infection and to an infection of the eye known as xerophthalmia.

A few years later evidence emanating from an inquiry carried out along entirely different lines from those employed in the researches on the fat-soluble A factor showed that another physiological function, namely, that of preventing rickets, might often be ascribed to this principle. Mellanby (1918, 1919, 1920, 1921), experimenting with puppies, demonstrated that rickets was a disease caused by dietary

deficiency. He was particularly struck by the fact that, although there were some discrepancies, the distribution of the factor which prevented rickets in puppies was very similar to that of the fat-soluble A factor as estimated on rats, an agreement sufficiently marked to warrant the assumption that a relationship existed between the two principles. The actual relationship was eventually disclosed. It was found that the antixerophthalmic or anti-infective function of the 'growth-promoting fat-soluble A factor' or 'vitamin A' was due to a principle distinct from the rickets-curing factor, although the two were closely associated in nature. After this differentiation, the former retained the original name of vitamin A, the latter became known as vitamin D. Each is capable of inducing growth but not in the absence of the other, and therefore all positive results obtained in investigations previous to the differentiation of the two vitamins, in which growth was the criterion, signify the presence of vitamins A and D together, whilst failure of growth in these cases might mean either the absence of both or one of these vitamins. Quantitative tests which disclose the presence of one fat-soluble vitamin, even if the other be absent, are now employed for vitamins A or D.

Butter, which is capable of inducing growth in rats fed on a diet deficient in both fat-soluble vitamins, contains therefore vitamins A and D, but in early investigations these two principles were assessed together as the 'growth-promoting fat-soluble A factor' or 'vitamin A'; only during the last few years were these vitamins studied in it differentially. Being an important article in the human dietary, butter naturally received the attention of many investigators in the field of vitamin research, and from these researches it soon became evident that there was a significant variation in the vitamin potency of butters. Steenbock, Boutwell, and Kent (1918), observing variations in the amount of the 'fat-soluble A factor' present in butter-fat, suggested that the ration of the cow might be responsible for these differences. Drummond and Coward (1920) and Drummond, Coward, and Watson (1921) showed that the variation was due to the difference in the diet of the cow and that butters produced during the winter months from stall-fed cows on dry feeds of hay, roots, and cake were of a low 'vitamin A' potency. Their experiments also indicated that the drying up of pasturage in a hot summer may lower the value of butter in this respect. Steenbock, Sell, and Buell (1921) found also that there was a seasonal variation in the vitamin content of butters; thus butters prepared at the end of March or April were markedly poorer in the 'fat-soluble A factor' than butters churned from milk of the same origin at the end of May or June. Hughes, Fitch, and Cave (1921) established a higher 'fat-soluble vitamin' content in the milk and butter from cows fed on a diet rich in this vitamin than in the milk produced by cows receiving a diet which was low in it. Kennedy and Dutcher (1922) observed that the presence of 'vitamin A' in cow's milk is entirely regulated by its occurrence in the ration of the animal and that stall-fed cows could produce a milk rich in vitamin if a proper combination of grain and leafy food was used.

Drummond *et al* (1923 and 1924) made similar observations and showed that the 'vitamin A' (i.e. vitamins A and D complex) could be raised by administering cod-liver oil, which is rich in vitamins A and D, to cows on winter rations. The administration of coco-nut oil or arachis oil, which are poor in the 'vitamin A complex' did not enhance the vitamin value of the butter. Luce (1924, 1 and 2) brought forward evidence that the diet of the cow was the principal factor in determining the growth-promoting and antirachitic properties of the milk. Chick and Roscoe (1926), estimating vitamin A (the anti-xerophthalmic or anti-infective vitamin) and vitamin D (the anti-rachitic vitamin) separately, found that the vitamin A content of the milk depended on the diet of the cow, being at a maximum when the animal was fed upon fresh, green fodder and least when the animal received a winter ration of cereals and roots. The milk contained the maximum amount of both fat-soluble vitamins A and D when the cow was at pasture in the summer. Golding, Soames, and Zilva (1926) have shown that the vitamin A, but not the vitamin D, content of the butter from cows fed on a winter ration, very poor in both fat-soluble vitamins, could be raised by the addition of kale. When cod-liver oil, however, was added to the diet of the cows instead of kale there was a very marked rise in both vitamins (A and D) in the butter. Wachtel (1929) and Steenbock, Hart, Hanning, and Humphrey (1930) found independently that the antirachitic potency of milk could be increased by feeding irradiated yeast to cows.

Another factor which possibly conduces to an increase of vitamin D but not vitamin A in milk is exposure of the animal to sunlight or to the ultra-violet radiation of a mercury quartz lamp. On this point the evidence is conflicting. Luce (1924) obtained no clear-cut evidence that irradiation of the cow increased the antirachitic properties of its milk. Chick and Roscoe (1926), however, found that the anti-rachitic value of milk depended on the degree of exposure of the cow to the sun. This finding was supported by the observation of Dutcher, Honeywell, and Dahle (1927), who reported that butter from cows subjected to sunlight possessed higher calcifying properties, and consequently antirachitic properties, than butter from cows kept in the dark. Falkenheim and co-workers (1926) did not observe an improvement in the antirachitic potency by insolating the cow, but found that its exposure to ultra-violet radiation increased the vitamin D content of the milk. The beneficial effect of ultra-violet irradiation of the cow on the vitamin D content of the milk was also recorded by Gowan and co-workers (1926) and by Bruck-Biesok, Pirquet, and Wagner (1927). On the other hand, Steenbock, Hart, Riising, Hoppert, Basherov, and Humphrey (1930) found that daily exposure of cows to sunlight or to artificial ultra-violet radiation had no demonstrable effect on the vitamin D of the milk. The antirachitic value of dried milk was also studied in this connexion by Hess and Unger (1921) and by Supplee and Dow (1927). Whilst the former workers found that dried summer milk was no better than dried 'stall-fed' milk in preventing the development or in decreasing the incidence of rickets in infants during

the winter, the latter observers established the superiority of the antirachitic property, as tested on rats, of dried summer milk over dried winter milk.

In spite of the conflicting nature of the above results, there is a general recognition that summer-produced milk and butter-fat is superior to the winter products. The primary cause of this may, as Steenbock and his collaborators suggest, lie in other factors than sunlight acting *directly* on the cow.

Concerning the stability of vitamins A and D in butter, the available evidence suggests that under satisfactory conditions of storage these vitamins do not deteriorate quickly. Osborne and Mendel (1916) observed that butter-fats kept at 18° C. in the dark and in the light and at 8° C. in the dark retained their 'vitamin A' activity for about 15 months. Steenbock, Boutwell, and Kent (1918) found that heated butters and butters kept in poor storage do not contain 'vitamin A'. Drummond, Coward, and Watson (1921) examined a number of samples of butter which had been in storage for various lengths of time (not longer than 8 months), usually at low temperatures, and found that they did not possess a lower average 'vitamin A' content than fresh samples. They concluded that butters stored below 10° C. do not suffer any appreciable loss of 'vitamin A' if undue exposure of surface to air is prevented and if other conditions of storage are good. The experiments of Chick and Roscoe (1926) showed that butter made from milk possessing antirachitic potency retained its activity after storage in a frozen condition for more than 2 years.

III. METHODS USED FOR ESTIMATING VITAMINS A AND D

A standard strain of the black and pied variety of the Norwegian rat was used in this investigation. The animals were bred at the Lister Institute by Miss H. H. Smith under conditions already described (Smith and Chick, 1926). A minor alteration in the diet was instituted in 1929, brown bread and marmite being replaced by dried yeast.

The cages in which the animals were kept during the test period consisted of a galvanized-iron, wire top $10 \times 10 \times 4$ in. and a tray $10\frac{3}{4} \times 10\frac{3}{4} \times 1$ in. The trays were filled with finely-broken peat over which rested a sheet of fine perforated zinc which served as a 'false-bottom'. In vitamin A tests each cage contained a glass jar which acted as a 'kennel' for the animal. Water was offered in inverted glass bulbs which, while making the water accessible to the rat, did not allow it to leak out. The bulbs were sterilized at the beginning of each test and the water was changed at least every other day. The peat was changed weekly and the 'false bottom' and trays were washed and disinfected at similar intervals. The temperature of the animal house was regulated by a thermostat and varied in the winter between 64° F. and 70° F. In the summer the temperature rose

occasionally to 80° F. but never fell below 65° F. The windows were coated with white paint and stippled, direct sunlight being scrupulously kept out.

1. Vitamin A.

This vitamin was estimated by a method based on Steenbock's principle. Rats were kept on a diet free from vitamin A but containing vitamin D, and at a stage when the bodily reserves of vitamin A of the animals began showing signs of depletion the test doses of the butters were administered. The growth response was taken as the chief criterion of vitamin A activity.

Rats 19–25 days old, weighing about 35 g. were employed in these tests. Each litter was kept in a large cage for the first 14 days, after which time they were placed in separate cages of the type described above. During the first month the animals received a diet, free from both fat-soluble vitamins A and D, which was constituted as follows:

Inactivated caseinogen	20 parts by weight.
Wheat starch	50 „ „ „
Hardened oil (cotton seed, sesame, or arachis)	15 „ „ „
Yeast, dried and powdered	8 (later 16) „
*Salt mixture	5 parts by weight.
Lemon-juice	5 „ „ „
Water	50 „ „ „

* NaCl	51·9
$MgSO_4.7H_2O$	164·0
$NaH_2PO_4.H_2O$	104·1
K_2HPO_4	286·2
$CaH_4(PO_4)_2.H_2O$	162·0
$Ca(C_3H_5O_3)_2.5H_2O$	390·0
Ferric citrate	35·4

The caseinogen, which was white and light in texture, was previously freed from traces of the fat-soluble vitamins by treatment in an electrically heated oven through which a current of air was circulating. The caseinogen was spread in thin layers on trays and heated for 51 hours at 115° C. During the working-hours of this period (about 21 hours), it was raked over hourly so that it was well exposed to the hot air. At the end of this treatment the caseinogen was buff-coloured and light. Care was taken to avoid overheating, since scorched, orange-coloured caseinogen causes diarrhoea in rats.

A month after the animals had been on the above experimental diet the hardened oil, before being incorporated, was irradiated in order to supply vitamin D. The fat was melted and poured into shallow, sheet-tin trays 30 × 50 cm. and exposed under a 'Pistolette' mercury-vapour quartz lamp (Hewittic Electric Co.) at a distance of 38 cm. for half an hour.

This diet was compounded about once a week and was kept at 0° C. in tightly closed containers. A weighed quantity of the diet was given daily to the individual rats. The quantity offered was always a few grammes in excess of that consumed the previous day, and the animals were thus supplied with the basal diet *ad lib.* Except in

vitamin A this basal diet was complete in all essential constituents, such as proteins, fats, carbohydrates, and salts; the yeast, lemon-juice, and irradiated oil supplied the vitamins B, C, and D respectively. Animals on this diet alone, after exhausting their bodily reserves of vitamin A, cease growing, lose weight, and ultimately die. A group of rats in each test was fed on the basal diet alone to test this for absence of vitamin A and served as negative controls. Another group, the positive controls, received the basal diet supplemented, during the test period, with 3 to 5 drops of cod-liver oil daily to provide an amount of vitamin A adequate to produce normal growth.

The rats were weighed three times a week; when they began showing premonitory symptoms of vitamin A depletion their weight was recorded daily until the depletion was well established, when the daily administration of the test dose began. This condition is usually manifested by a loss of appetite, muscular tone, glossiness of coat, &c. Though eye symptoms usually develop, they are not invariable. On the other hand, eye symptoms may be present while the animal is still growing. The rats were carefully watched for any of the above symptoms after having subsisted about 4 weeks on the basal diet. The exact time for starting the dosage with the test substance can only be gauged by experience and it is difficult to lay down hard and fast rules. The following main indications were, however, adopted: (*a*) when the weight had been stationary for some time, (*b*) when a sudden considerable loss of weight (5–10 g.), especially following on a short stationary period, occurred, and (*c*) when gradual loss of weight followed a short period of weight maintenance.

A quantity containing 7 doses of the test butter was weighed out weekly for each animal and kept in the dark at 0° C. From this store about one-sixth was removed daily, except on Sundays, and administered to the rat, precautions being taken that the butter was entirely consumed. The administration of the dose was continued for 4 weeks.

Each test group consisted of 6 animals, 3 of each sex, and as the growth response in rats varies not only with sex but also with litter, members of each family were distributed among the groups receiving the test doses and those acting as positive or negative controls; these groups were, therefore, as evenly balanced as possible.

2. Vitamin D.

The Chick, Korenchevsky, and Roscoe method (1926) was employed. In this method the rats receive a basal diet low in the antirachitic vitamin and in phosphorus, whilst the test doses are administered from the beginning of the test. The degree of calcification in the bone as determined by the ash content is used as a criterion for vitamin D activity.

The rats were started on the test when 21 to 25 days old (weight about 42 g.) and were uniformly distributed as regards sex and litter among the various groups receiving the different doses (at least 6

rats per group). They received McCollum diet 3143, which is composed as follows:

Wheat	33	parts by weight.	
Maize	33	„	„
Wheat gluten	15	„	„
Gelatin	15	„	„
Calcium carbonate	3	„	„
Sodium chloride	1	„	„

The gelatin was heated in 60 c.c. of water and mixed thoroughly with the other components and the whole allowed to set in a cake. The diet was made up about once a week and stored in a tightly closed container at 0° C. The animals were offered the diet *ad lib.*; the administration of it and of the test-butter doses and the weighing of the uneaten food residues were carried out in the same way as in the vitamin A tests. The rats in the positive control groups subsisted on the basal diet plus 3 to 5 drops of cod-liver oil per day, and those in the negative control groups on the basal diet alone.

The animals were killed by coal gas after 28 days and both hind-legs were removed within a few hours of death and carefully cleaned from adhering flesh and the patellae. The bones (femur, tibia, and fibula) were weighed in weighing bottles, heated in an oven at 105° C. for 24 hours and the dry weight determined. They were then tied up in fine-meshed silk free from flaws and broken between the fingers in order to ensure better penetration of the solvent. The silk bags and the contents were next extracted in boiling absolute alcohol under a reflux condenser for 8 hours and allowed to remain in the alcohol overnight. After a 24-hours ether extraction in a Soxhlet apparatus and drying in an oven for 4 hours at 105° C., the 'fat-free' weight was obtained and the ash content determined by incineration. The degree of calcification of the bone was judged by the relative ash content or by the relative value of the ratio A/R, i.e. the ratio of bone ash to 'organic residue'. The latter was obtained by subtracting the weight of the ash from the weight of the dry, extracted bone.

3. The Statistical Criterion Employed.

The original intention was to determine the relative potencies of the butters by the minimum protective dose, but the small number of animals (6 per dose) and the relatively few doses employed in each test made this a not very reliable comparison. Recourse was, therefore, had to the additional aid of a statistical method. The statistical examination of our results was carried out by Miss H. M. Woods, F.S.S., from the Division of Epidemiology, London School of Hygiene and Tropical Medicine, to whom we should like to express our deep gratitude for the help she has rendered us.

The number of animals used in each experimental group was small, and for statistical examination it was thought that Fisher's Test (Fisher, 1930), known as the 't' test for measuring the significance of differences of means in small samples, was the most appropriate method to apply. In this method the standard deviation is estimated

by pooling the sum of the squares of the deviations from the mean in the two samples and dividing by the total number of degrees of freedom contributed by them. 't' is obtained by calculating the ratio of the differences between the means of the two samples to the standard error as estimated. Fisher has tabulated the 5 per cent. and 1 per cent. values of 't' such that fluctuations of sampling would only give rise to as large or larger values once in twenty and once in a hundred times respectively. The 5 per cent. test has been used as a criterion of significance throughout this study. For example, to test the significance of a gain in weight, the value of 't' was determined for the two samples, experimental and positive control, and if this was exceeded by the 5 per cent. value of 't' in Fisher's tables for N-2, i.e. allowing two degrees of freedom, the effect of diet on growth was regarded as not significantly different in the two samples. When the results from the two groups differ significantly it is very unlikely that this difference is due to chance, and probable (20 chances to 1) that the divergence is due to the difference of treatment of the groups, i.e. that the doses given were markedly different in potency. When the results from two groups although different are not significantly so, then this difference may have been due to chance and it would be rarely justifiable to assume a definite difference in the potency of the doses under comparison.

In each vitamin A experiment the mean daily gain in weight during the period before and after addition of butter to the diet has been compared with that of control animals receiving cod-liver oil, in order to see whether any difference in treatment had had a significant effect. In the few cases where in the preparatory period the average growth of a group differed significantly from the controls this showed that the two groups were not comparable as regards growth in the preparatory period, and it is difficult to draw definite conclusions from the growth of the rats after the dose of vitamin had been added to the diet.

In the vitamin D tests the statistical interpretation of the results relies on the A/R ratio as determined from the leg-bones of the rats at the end of the experiment.

IV. NEW ZEALAND BUTTERS

We are indebted to Mr. W. Wright, Inspector of Dairy Products of the Dominion of New Zealand, for the following general details concerning the preparation of butter in New Zealand.

In New Zealand the cows are on pasture all the year round. Practically the entire milk intended for butter-making is skimmed on the farm and the cream transported to the factory in the majority of cases every second day and in some instances every day. With the exception of 4 or 5 proprietary places these butter factories are worked on a co-operative basis. On arrival at the factories the cream is tested for acidity, which of course varies with the season and the prevailing temperature, and if necessary is neutralized with sodium bicarbonate.

rats per group). They received McCollum diet 3143, which is composed as follows:

Wheat	.	.	.	.	33	parts by weight.
Maize	.	.	.	.	33	„ „ „
Wheat gluten	.	.	.	.	15	„ „ „
Gelatin	.	.	.	.	15	„ „ „
Calcium carbonate	.	.			3	„ „ „
Sodium chloride	.	.			1	„ „ „

The gelatin was heated in 60 c.c. of water and mixed thoroughly with the other components and the whole allowed to set in a cake. The diet was made up about once a week and stored in a tightly closed container at 0° C. The animals were offered the diet *ad lib.*; the administration of it and of the test-butter doses and the weighing of the uneaten food residues were carried out in the same way as in the vitamin A tests. The rats in the positive control groups subsisted on the basal diet plus 3 to 5 drops of cod-liver oil per day, and those in the negative control groups on the basal diet alone.

The animals were killed by coal gas after 28 days and both hind-legs were removed within a few hours of death and carefully cleaned from adhering flesh and the patellae. The bones (femur, tibia, and fibula) were weighed in weighing bottles, heated in an oven at 105° C. for 24 hours and the dry weight determined. They were then tied up in fine-meshed silk free from flaws and broken between the fingers in order to ensure better penetration of the solvent. The silk bags and the contents were next extracted in boiling absolute alcohol under a reflux condenser for 8 hours and allowed to remain in the alcohol overnight. After a 24-hours ether extraction in a Soxhlet apparatus and drying in an oven for 4 hours at 105° C., the 'fat-free' weight was obtained and the ash content determined by incineration. The degree of calcification of the bone was judged by the relative ash content or by the relative value of the ratio A/R, i.e. the ratio of bone ash to 'organic residue'. The latter was obtained by subtracting the weight of the ash from the weight of the dry, extracted bone.

3. The Statistical Criterion Employed.

The original intention was to determine the relative potencies of the butters by the minimum protective dose, but the small number of animals (6 per dose) and the relatively few doses employed in each test made this a not very reliable comparison. Recourse was, therefore, had to the additional aid of a statistical method. The statistical examination of our results was carried out by Miss H. M. Woods, F.S.S., from the Division of Epidemiology, London School of Hygiene and Tropical Medicine, to whom we should like to express our deep gratitude for the help she has rendered us.

The number of animals used in each experimental group was small, and for statistical examination it was thought that Fisher's Test (Fisher, 1930), known as the 't' test for measuring the significance of differences of means in small samples, was the most appropriate method to apply. In this method the standard deviation is estimated

by pooling the sum of the squares of the deviations from the mean in the two samples and dividing by the total number of degrees of freedom contributed by them. 't' is obtained by calculating the ratio of the differences between the means of the two samples to the standard error as estimated. Fisher has tabulated the 5 per cent. and 1 per cent. values of 't' such that fluctuations of sampling would only give rise to as large or larger values once in twenty and once in a hundred times respectively. The 5 per cent. test has been used as a criterion of significance throughout this study. For example, to test the significance of a gain in weight, the value of 't' was determined for the two samples, experimental and positive control, and if this was exceeded by the 5 per cent. value of 't' in Fisher's tables for N-2, i.e. allowing two degrees of freedom, the effect of diet on growth was regarded as not significantly different in the two samples. When the results from the two groups differ significantly it is very unlikely that this difference is due to chance, and probable (20 chances to 1) that the divergence is due to the difference of treatment of the groups, i.e. that the doses given were markedly different in potency. When the results from two groups although different are not significantly so, then this difference may have been due to chance and it would be rarely justifiable to assume a definite difference in the potency of the doses under comparison.

In each vitamin A experiment the mean daily gain in weight during the period before and after addition of butter to the diet has been compared with that of control animals receiving cod-liver oil, in order to see whether any difference in treatment had had a significant effect. In the few cases where in the preparatory period the average growth of a group differed significantly from the controls this showed that the two groups were not comparable as regards growth in the preparatory period, and it is difficult to draw definite conclusions from the growth of the rats after the dose of vitamin had been added to the diet.

In the vitamin D tests the statistical interpretation of the results relies on the A/R ratio as determined from the leg-bones of the rats at the end of the experiment.

IV. NEW ZEALAND BUTTERS

We are indebted to Mr. W. Wright, Inspector of Dairy Products of the Dominion of New Zealand, for the following general details concerning the preparation of butter in New Zealand.

In New Zealand the cows are on pasture all the year round. Practically the entire milk intended for butter-making is skimmed on the farm and the cream transported to the factory in the majority of cases every second day and in some instances every day. With the exception of 4 or 5 proprietary places these butter factories are worked on a co-operative basis. On arrival at the factories the cream is tested for acidity, which of course varies with the season and the prevailing temperature, and if necessary is neutralized with sodium bicarbonate.

It is then pasteurized and cooled. The churning takes place the following day, and the butter is packed and forwarded to the grading store. After grading it is transferred to cold-store where it remains until it is shipped, usually not longer than a fortnight. All butters must remain in cold store for at least four days before shipment. The transit to this country takes 32 to 42 days, during which time the butter is kept at about 15° F. The bulk of the consignments reach the consumer within a short period after the discharge of the goods, and those consignments which are not sold immediately after arrival are kept in cold store (about 15° F.) before reaching the retailer.

The butters investigated in these experiments were prepared at Lepperton and Mangorei, on the northern slopes of Mount Egmont, near New Plymouth, Taranaki, during the summer of 1927–8 and the winter of 1929. The following are the details of the origin and preparation of the experimental material. The method of preparation is representative of that adopted in New Zealand in the manufacture of creamery butter for export to the United Kingdom and also for local consumption.

1. Summer Butters, 1927–8.

A. Methods of Preparation.

At Mangorei the butters were prepared from mixed breeds and at Lepperton from Jersey cows only. They were made at the height of the grass period, namely, 22 and 24 December 1927. No food was offered to supplement the pasture. New Plymouth sunshine records covering 20 days prior to the cream being selected are as follows:

1927.	*Bright Sunshine.* Hours.	Minutes.
December 2	12	50
„ 4	12	55
„ 5	13	5
„ 6	0	50
„ 7	5	5
„ 8	9	35
„ 9	12	35
„ 11	11	0
„ 12	10	55
„ 13	9	0
„ 14	5	40
„ 15	8	20
„ 16	9	5
„ 18	5	20
„ 19	9	10
„ 20	7	0
„ 21	10	15
„ 22	8	5

The average 'bright sunshine' per day equalled 8 hrs. 56 min. The average lactation state of the herds from which the cream selected was drawn was 5 months. The soil and climatic conditions of the districts surrounding the factories selected were comparable. Details of cream selected and manufactured are as follows:

Lepperton butters.

The Lepperton factory was selected for making the butter from cream of a pure Jersey herd. The cream used was graded as 'finest'. The parcels of cream separated on the farm selected were thoroughly mixed together, and then halved.

Jersey not neutralized. The first half was made into 'not neutralized' butter; details of manufacture are as follows:

Acidity of cream before pasteurizing . .	0·18% as lactic acid.

The cream was pasteurized on the 'flash' system in two stages. The pasteurizing temperature was at the 1st stage 168° F., and the 2nd stage 204° F. The cream was immediately cooled by a water cooler.

Temperature of cream off cooler . .	74° F.
Acidity of cream off cooler . . .	0·14%

No starter was used.

The cream was then held in a refrigerated vat overnight for 17 hours before churning.

Churning temperature	47° F.
Churning acidity at time of putting in cream . . .	0·14%
Temperature of wash water	39° F.

A combined churn and worker was used.

The butter was graded 94½ points.

Jersey neutralized. The remaining half of the selected parcels of cream separated on the farm was made up into 'neutralized' butter; the details of manufacture are as follows:

Acidity of cream when halved from the selected bulk .	0·18%

Acidity was raised by adding starter and allowing to ripen.

Acidity of cream before neutralizing	0·25%

Bicarbonate of soda was used as the neutralizing agent.

The cream was pasteurized on the 'flash' system in two stages, 1st stage 180° F., 2nd stage 204° F.

Temperature of cream off water cooler	63° F.
Acidity of cream off cooler	0·11%
Acidity reduction effected by neutralizing and pasteurizing	0·14%

The cream was held overnight in a refrigerated vat for 16 hours.

Churning temperature	46° F.
Churning acidity	0·12%
Temperature of wash water . . .	39°F.

The butter was graded 94½ points.

Mangorei butters.

All cream used was graded as 'finest'. 3,242 lb. of cream were used. The percentages of cream used from the various breeds of cows are as follows:

Ayrshire	15%
Shorthorn Cross . . .	15%
Holstein	34%
Jersey	36%

The parcels of cream separated on the farm selected were thoroughly mixed together and then halved.

Mixed breeds not neutralized. The first half was made up into 'not neutralized' butter, and details of manufacture were as follows:

Acidity of cream before pasteurizing	0·17%

The cream was pasteurized on the 'flash' system in two stages, 1st stage 180° F., 2nd stage 200° F. and was immediately cooled, first by a water cooler and secondly by a direct expansion refrigerated cooler.

Temperature of cream off refrigerated cooler	54° F.
Acidity of cream off cooler	0·14%

No starter was used.

The cream was then held in a refrigerated vat overnight for 16 hours before churning.

Churning temperature	46° F.
Churning butter-fat content	35%
Churning acidity	0·15%
Temperature of wash water	39° F.

A combined churn and worker was used.

The butter was graded 94 points.

Mixed breeds neutralized. The remaining half of the selected parcels of cream separated on the farm was made up into 'neutralized' butter; the details of manufacture are as follows:

Acidity of cream when halved from the selected bulk	0·17%

Acidity was raised by adding starter and allowing to ripen.

Acidity of cream before neutralizing	0·21%

Bicarbonate of soda was used as the neutralizing agent.

The cream was pasteurized on the 'flash' system in two stages, 1st stage 180° F., 2nd stage 200° F.

Temperature off refrigerated cooler	53° F.
Acidity of cream off cooler	0·12%
Acidity reduction effected by neutralizing and pasteurizing	0·09%

The cream was held overnight in a refrigerated vat for 14 hours.

Churning temperature	44° F.
Churning butter-fat content	33%
Churning acidity	0·13%
Temperature of wash water	40° F.

The butter was graded 94 points.

Both Lepperton and Mangorei butters were held in the factory cool chambers at approximately 40° F. for a few days, before being forwarded with other lines of butter to the Moturoa grading stores.

These butters reached the laboratory on 10 March 1928. The test for vitamin A was carried out in March to June 1928. Vitamin D was determined in the 'Jersey neutralized' and 'Mixed breeds neutralized' butters in April to May and in the 'Jersey not neutralized' and 'Mixed breeds not neutralized' in May to June 1928.

B. Results of Tests.

(*a*) *Vitamin A.*

In these tests 3·3 per cent. marmite, calculated on the wet weight, was used in the basal diet as a source of the vitamin B complex. Later researches (Collison, Hume, Smedley-Maclean, and Smith, 1929) have suggested, however, that this might not be quite adequate and consequently there is a possibility that the growth of the rats may have been somewhat limited. This should be borne in mind while considering the results of this test.

Jersey not neutralized (Table 1).

Although in the preparatory period the rate of growth was the highest in the positive control group, there was no significant difference in this respect between it and that of the animals receiving 0·6 g. and 0·1 g. doses. After dosing, the response by the rats receiving 0·6 g. or 0·2 g., although less marked than that of the control group, did not differ from it significantly. The lower rate of growth of the rats on the lowest dose did differ significantly. As the animals on the 0·2 g. dose were unsatisfactory, in so far as their growth in the preparatory period was low, it is only possible to say that the minimum curative dose did not lie below 0·1 g.

Jersey neutralized (Table 1).

As in the previous test the rate of growth in the preparatory period was highest in the positive control group; there was, however, no significant difference between it and the groups on the two highest doses. After dosing, the response in all groups was less marked than that of the control but the discrepancy was not statistically significant. As the animals in 0·1 g. group were unsatisfactory and as the difference between the 0·2 g. group and the positive controls was nearly significant, the minimum curative dose probably lies in the neighbourhood of 0·2 g.

Mixed breeds neutralized (Table 1).

In the preparatory period all groups did not grow as well as the control group, but only in the 0·2 g. group was the difference statistically significant. After dosage, the lower response was not significant in the 0·6 g. group, but was significant in the 0·2 g. and 0·1 g. groups. It is difficult to interpret the results obtained with 0·2 g. since the growth of this group also deviated from that of the control in the preparatory period. It is only possible to say that the minimum dose does not lie below 0·1 g.

Mixed breeds not neutralized (Table 1).

In this test the difference in rate of growth between the control group and the other groups in the preparatory period was statistically

significant only in the 0·2 g. group. After dosing, however, this difference was only significant in the 0·1 g. group. It is therefore only possible to give the same assessment as for the preceding butter.

TABLE 1.

Influence of method of preparation and herd on the vitamin A content of New Zealand butters, summer 1927–8.

	Average daily rate of growth.	*N-2.*	*Value of t.*	*5 per cent. value of t.*	
Preparatory period.					
Positive controls . . .	1·04 g.				
Jersey not neutralized.					
0·6 g.	0·93 g.	14	−1·11	2·15	not sig.
0·2 „	0·77 „	15	−3·81	2·13	sig.
0·1 „	0·96 „	13	−0·85	2·16	not sig.
Jersey neutralized.					
0·6 g.	0·95 g.	13	−0·89	2·16	not sig.
0·2 „	0·92 „	14	−1·06	2·15	not sig.
0·1 „	0·86 „	14	−2·64	2·15	sig.
Mixed breeds neutralized.					
0·6 g.	0·95 g.	14	−1·00	2·15	not sig.
0·2 „	0·86 „	14	−2·48	2·15	sig.
0·1 „	0·96 „	14	−0·96	2·15	not sig.
Mixed breeds not neutralized.					
0·6 g.	0·88 g.	14	−1·79	2·15	not sig.
0·2 „	0·84 „	14	−2·49	2·15	sig.
0·1 „	0·90 „	14	−1·47	2·15	not sig.
Dosing period.					
Positive controls . . .	1·23 g.				
Jersey not neutralized.					
0·6 g.	0·81 g.	14	−1·67	2·15	not sig.
0·2 „	0·99 „	15	−1·09	2·13	not sig.
0·1 „	0·37 „	13	−2·80	2·16	sig.
Jersey neutralized.					
0·6 g.	1·05 g.	13	−0·73	2·16	not sig.
0·2 „	0·78 „	14	−2·06	2·15	not sig.
0·1 „	0·74 „	14	−1·95	2·15	not sig.
Mixed breeds neutralized.					
0·6 g.	1·00 g.	14	−1·04	2·15	not sig.
0·2 „	0·62 „	14	−2·40	2·15	sig.
0·1 „	0·55 „	14	−3·12	2·15	sig.
Mixed breeds not neutralized.					
0·6 g.	0·97 g.	14	−1·16	2·15	not sig.
0·2 „	0·86 „	14	−1·50	2·15	not sig.
0·1 „	0·50 „	14	−3·02	2·15	sig.

(b) Vitamin D.

It will be seen (Table 2) that the maximum A/R ratio was obtained in each case with a daily dose of 0·6 g. of butter, whilst with the 0·2 g.

and still more with the 0·1 g. doses lower figures were registered. The differences recorded in the case of the lower doses between the various butters are, in our opinion, of such an order as to fall within the limits of the experimental error of the method and consequently they cannot be taken as a significant index of unequal vitamin D contents.

TABLE 2.

Influence of method of preparation and herd on the vitamin D content of New Zealand butters, summer 1927–8.

	Average A/R.	*Degree of protection.*	*N-2.*	*Value of t.*	*5 per cent. value of t.*	
Positive controls. . . .	0·76					
Jersey neutralized.						
0·6 g.	0·80	117	10	+0·93	2·23	not sig.
0·2 g.	0·70	74	10	−2·46	2·23	sig.
0·1 „	0·65	52	10	−2·17	2·23	not sig.
Mixed breeds neutralized.						
0·6 g.	0·79	113	10	+0·98	2·23	not sig.
0·2 „	0·70	74	10	−1·35	2·23	not sig.
0·1 „	0·67	61	10	−2·18	2·23	not sig.
Negative controls . . .	0·53					
Positive controls . . .	0·79					
Jersey not neutralized.						
0·6 g.	0·81	109	9	+0·32	2·26	not sig.
0·2 „	0·75	83	8	−0·98	2·31	not sig.
0·1 „	0·62	26	8	−4·71	2·31	sig.
Mixed breeds not neutralized.						
0·6 g.	0·80	104	9	+0·13	2·26	not sig.
0·2 „	0·69	57	9	−3·01	2·26	sig.
0·1 „	0·67	48	9	−2·67	2·26	sig.
Negative controls . . .	0·56.					

In this and in all subsequent Tables of vitamin D tests:

A/R = Ash/ 'organic residue'

$$\text{Degree of protection} = \frac{D-N}{P-N} \times 100$$

where:

D = Average A/R of dose group.
N = „ „ „ „ negative control group.
P = „ „ „ „ positive control group.

2. Stored Summer Butters.

RESULTS OF TESTS.

(*a*) *Vitamin A.*

The summer butters were re-tested for vitamin A in March to July 1930, namely, 2 years after arrival in England. During this period the experimental material was kept in cold-store at 9–13° F.

The basal diet differed slightly from the one used when these

butters were first tested. As a source of the vitamin B complex, 5 per cent. and eventually 10 per cent. of dried yeast, calculated on the wet weight of the diet, was used instead of 3·3 per cent. marmite. This alteration, as already mentioned, was effected because, during the interval between the tests, indications were obtained that the source of the vitamin B complex used in the first test was not quite satisfactory.

TABLE 3.

Influence of storage on the vitamin A content of New Zealand butters, summer 1927–8.

	Average daily rate of growth.	*N-2.*	*Value of t.*	*5 per cent. value of t.*	
Preparatory period.					
Positive controls . . .	1·09 g.				
Jersey not neutralized.					
0·2 g.	1·00 g.	11	−0·48	2·20	not sig.
0·1 „	1·15 „	11	+0·35	2·20	not sig.
Jersey neutralized.					
0·2 g.	1·25 g.	11	+0·73	2·20	not sig.
0·1 „	1·08 „	11	−0·06	2·20	not sig.
Mixed breeds neutralized.					
0·2 g.	1·26 „	11	+0·88	2·20	not sig.
0·1 „	1·10 „	12	+0·06	2·18	not sig.
Mixed breeds not neutralized.					
0·2 g.	1·07 g.	11	−0·11	2·20	not sig.
0·1 „	1·19 „	11	+0·59	2·20	not sig.
Dosing period.					
Positive controls . . .	2·68 g.				
Jersey not neutralized.					
0·2 g.	1·69 g.	11	−2·44	2·20	sig.
0·1 „	0·69 „	11	−6·65	2·20	sig.
Jersey neutralized.					
0·2 g.	1·77 g.	11	−3·22	2·20	sig.
0·1 „	1·24 „	11	−5·40	2·20	sig.
Mixed breeds neutralized.					
0·2 g.	1·92 g.	11	−3·41	2·20	sig.
0·1 „	1·37 „	12	−5·47	2·18	sig.
Mixed breeds not neutralized.					
0·2 g.	1·74 g.	11	−2·61	2·20	sig.
0·1 „	1·38 „	11	−4·66	2·20	sig.

The response in growth of the positive control group was exceptionally high (Table 3). In these tests the growth response of the two doses tested (0·2 g. and 0·1 g.) was higher in every case than in the tests carried out 2 years previously (Table 1). Also, on the whole, the time taken to deplete the rats of vitamin A was shorter. This was most probably due to the change of the source and level of the

vitamin B complex in the basal diet. As will be seen from Table 3, in all cases the highest dose tested (0·2 g.), did not give as good a growth response as the cod-liver oil in the positive control group; moreover, this difference was statistically significant. It must be noted, however, that the response in growth of the positive control group was exceptionally high in this test. In the preparatory periods there was no significant difference between the rate of growth in the butter groups and in the positive control group. The general indication points to the fact that no loss of vitamin A took place during storage.

(*b*) *Vitamin D.*

The re-tests took place in March to May 1930, after nearly two years storage. The results are contained in Table 4. It is evident from these data that all the butters showed less protection in daily doses of 0·2 g. after storage than before (Table 2). The higher dose (0·6 g.) gave, within the limits of the experimental error of the method, complete protection.

TABLE 4.

Influence of storage on the vitamin D content of New Zealand butters, summer 1927–8.

	Average A/R.	*Degree of protection.*	*N-2.*	*Value of t.*	*5 per cent. value of t.*	
Positive controls . . .	0·80					
Jersey not neutralized.						
0·6 g.	0·79	96	11	−0·28	2·20	not sig.
0·2 „	0·59	22	11	−3·76	2·20	sig.
Jersey neutralized.						
0·6 g.	0·80	100	11	−0·03	2·20	not sig.
0·2 „	0·60	26	11	−3·76	2·20	sig.
Mixed breeds neutralized.						
0·6 g.	0·77	89	11	−0·85	2·20	not sig.
0·2 „	0·59	22	11	−3·38	2·20	sig.
Mixed breeds not neutralized.						
0·6 g.	0·78	93	11	−0·34	2·20	not sig.
0·2 „	0·64	41	11	−2·91	2·20	sig.
Negative controls . . .	0·53					

It is to be noted that the antirachitic potency of the four butters differed somewhat before but not after storage. There is little doubt that all these butters lost some vitamin D during storage.

3. Winter Butters, 1929.

A. Origin and Methods of Preparation.

As in the previous experiment, butters were prepared from mixed breeds at Mangorei and from Jersey cows only at Lepperton. The butters were made on 9 May and 16 May 1929, these dates being as

near to mid-winter as it was possible to go on account of the very small supply of cream available in the month of June. No feed was offered to supplement the pasture feeding. The average lactation period of the supplying herds on 15 May was 2½ months. Sunshine records for the district for 36 days prior to the butters being made are as follows:

1929.	*Bright Sunshine. Hours.*
April 1	10
,, 2	9
,, 3	10
,, 4	10
,, 5	10
,, 7	11
,, 8	5
,, 9	1
,, 10	10
,, 11	1
,, 12	1
,, 14	Nil
,, 15	6
,, 16	Nil
,, 17	6
,, 18	2
,, 19	9
,, 21	8
,, 22	4
,, 23	5
,, 25	2
,, 26	9
,, 28	3
,, 29	1
,, 30	6
May 1	7
,, 2	8
,, 3	1
,, 5	9
,, 6	8
,, 7	9

The average sunshine per day was 6 hours. Climatic conditions were good for 6 weeks prior to the butter being made, moderate weather and warm rains being experienced. The climatic conditions were comparable, as the two factories are only a few miles apart. Details of cream selected and manufactured are as follows:

Lepperton butters.

Cream from a purely Jersey herd of cows was used at the Lepperton Factory. Approximately 95 per cent. of the cream used was graded as 'finest', the balance being '1st grade'. The parcels of cream were thoroughly mixed, then halved.

Jersey not neutralized. The first half was made up into 'not neutralized' butter; details of manufacture are as follows:

Acidity of cream before pasteurizing . . . . 0·17%
No neutralizing agent was used.

The cream was pasteurized by the 'flash' system in two stages to a final temperature of 204° F.

Temperature of cream off water cooler	64° F.
Acidity of cream off cooler	0·14%

The cream was held overnight in a refrigerated vat for 16 hours before churning.

Churning temperature	50° F.
Churning acidity	0·15%
Temperature of wash water	41° F.

Analysis of butter:

Butter-fat	81·7%
Water	15·3%
Salt	2·2%
Curd and ash	0·8%

The butter was graded 93½ points.

Jersey neutralized. The remaining half of the selected cream was made into 'neutralized' butter, the details of manufacture are as follows:

Initial acidity of cream	0·17%

Sodium bicarbonate was used as the neutralizing agent. The cream was pasteurized by the 'flash' system in two stages to a final temperature of 204° F.

Temperature of cream off water cooler	65° F.
Acidity of cream off cooler	0·11%
Acidity reduction effected by neutralizing and pasteurizing	0·06%

The cream was held overnight in a refrigerated vat for 15 hours before churning.

Churning temperature	46° F.
Churning acidity	0·13%
Temperature of wash water	40° F.

Analysis of butter:

Butter-fat	82·5%
Water	15·4%
Salt	1·6%
Curd and ash	0·5%

The butter was graded 93½ points.

Mangorei butters.

Approximately 94 per cent. of the cream used was graded as 'finest', the balance being classed as '1st grade'. The following breeds of cows were well represented in the cream used: Ayrshire, Holstein, Shorthorn Cross and Jersey. The selected cream was divided into two equal batches.

Mixed breeds not neutralized. Half the selected cream was made up into 'not neutralized' butter; the details of manufacture are as follows:

Acidity of cream before pasteurizing and after division	0·16%

No neutralizing agent was used.

The cream was pasteurized by the 'flash' system in two stages to a final temperature of 200° F.

Temperature of cream off refrigerated cooler	51° F.
Acidity of cream off cooler	0·13%

The cream was held overnight in a refrigerated vat for 15 hours before churning.

Churning temperature	43° F.
Churning acidity	0·14%
Temperature of wash water	40° F.

Analysis of butter:

Butter-fat	81·4%
Water	15·4%
Salt	2·4%
Curd and ash	0·8%

The butter was graded 93½ points.

Mixed breeds neutralized. The remaining batch of the selected cream was made into 'neutralized' butter; details of manufacture were as follows:

Acidity of cream (after division)	0·17%

Sodium bicarbonate was used as the neutralizing agent. The cream was pasteurized by the 'flash' system in two stages to a final temperature of 200° F.

Temperature of cream off refrigerated cooler	51° F.
Acidity of cream off cooler	0·07%
Acidity reduction effected by neutralizing and pasteurizing	0·1%

The cream was held overnight in a refrigerated vat for 14 hours before churning.

Churning temperature	46° F.
Churning acidity	0·08%
Temperature of wash water	40° F.

Analysis of butter:

Butter-fat	81·7%
Water	15·4%
Salt	2·1%
Curd and ash	0·8%

The butter was graded 93½ points.

Both Lepperton and Mangorei butters were held in the factory cool chambers at approximately 40° F. for a few days before being forwarded to the freezing works for grading and storage, prior to shipment.

The butters reached the laboratory on 15 August 1929. The tests for vitamin A were carried out in October to November 1929. Vitamin D was determined during September to October, 1929.

B. Results of Tests.

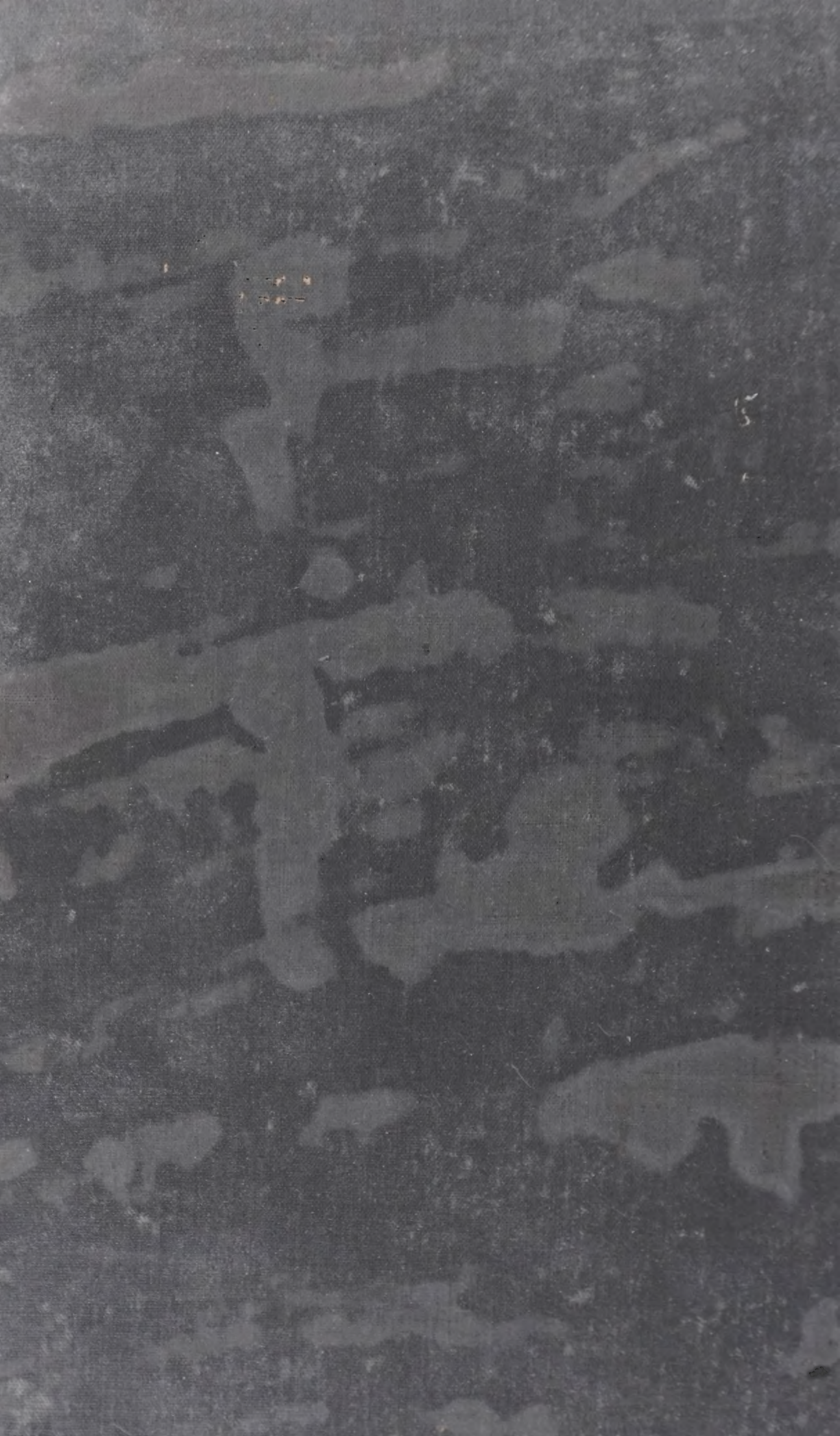
(*a*) *Vitamin A.*

Five per cent. dried yeast, calculated on the wet weight, as the source of the vitamin B complex, and hardened sesame oil were used in the basal diet in this test.

Jersey not neutralized (Table 5).

The small differences in the rate of growth between all groups and that of the positive control group before dosing were not significant. After dosage, the response in all groups was less than that of the control group and only in the 0·2 g. group was this difference not significant. One may, therefore, assume that the minimum dose was probably between 0·1 g. and 0·2 g. and consequently the activity of this butter is of the same order as that of the summer butter.

TABLE 5.

Influence of method of preparation and herd on the vitamin A content of New Zealand butters, winter 1929.

	Average daily rate of growth.	*N-2*	*Value of t.*	*5 per cent. value of t.*	
Preparatory period.					
Positive controls	1·12 g.				
Jersey not neutralized.					
0·2 g.	1·13 g.	13	+0·08	2·16	not sig.
0·1 „	1·38 „	13	+1·61	2·16	not sig.
0·05 „	1·22 „	13	+0·73	2·16	not sig.
Jersey neutralized.					
0·2 g.	1·24 g.	13	+1·00	2·16	not sig.
0·1 „	1·21 „	13	+0·63	2·16	not sig.
0·05 „	1·25 „	13	+1·07	2·16	not sig.
Mixed breeds neutralized.					
0·2 g.	1·14 g.	13	+0·14	2·16	not sig.
0·1 „	1·28 „	13	+1·15	2·16	not sig.
0·05 „	1·30 „	13	+1·35	2·16	not sig.
Mixed breeds not neutralized.					
0·2 g.	1·27 g.	13	+1·02	2·16	not sig.
0·1 „	1·28 „	13	+1·16	2·16	not sig.
0·05 „	1·29 „	13	+1·16	2·16	not sig.
Dosing period.					
Positive controls	1·17 g.				
Jersey not neutralized.					
0·2 g.	0·86 g.	13	−1·42	2·16	not sig.
0·1 g.	0·70 „	13	−4·22	2·16	sig.
0·05 „	0·40 „	13	−7·55	2·16	sig.
Jersey neutralized.					
0·2 g.	0·65 g.	13	−3·07	2·16	sig.
0·1 „	0·73 „	13	−3·72	2·16	sig.
0·05 „	0·15 „	13	−7·44	2·16	sig.
Mixed breeds neutralized.					
0·2 g.	0·09 g.	13	−3·35	2·16	sig.
0·1 „	0·71 „	13	−1·70	2·16	not sig.
0·05 „	0·10 „	13	−4·02	2·16	sig.
Mixed breeds not neutralized.					
0·2 g.	0·96 g.	13	−1·29	2·16	not sig.
0·1 „	−0·11 „	13	−4·76	2·16	sig.
0·05 „	−0·50 „	13	−4·80	2·16	sig.